REVIEW

Leflunomide: a possible alternative for gangciclovir sensitive and resistant cytomegalovirus infections

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The search for newer more cost effective treatments for infectious diseases remains a challenge. Cytomegalovirus (CMV) infection, which is especially common in the immunosupressed, is an important challenge for treating physicians. Gangciclovir's cost is a major hurdle in developing countries. Leflunomide is cheaper and is easily given orally. It works by a novel mechanism inhibiting virion assembly. It also has immunosuppressive properties. It and has been shown to be effective in both gangciclovir sensitive as well as resistant cases of CMV infection. Given these considerations we believe that leflunomide is an exiting new drug for CMV infection. However, hepatotoxicity and teratogenicity are known side effects. The exact dose and duration of treatment for CMV infection, for secondary prophylaxis, and in situations of gangciclovir resistance need further study.

With the advent of increasing immunosupression and the HIV pandemic cytomegalovirus (CMV) infection has become common.[1] It is an important cause of morbidity and mortality among transplant recipients, frequently engaging the clinician in a struggle to balance graft preservation with control of CMV disease. The treatment of choice has been gangciclovir[2] given parenterally. However, it is expensive and requires a trained medical person to administer. Gangciclovir resistance is being increasingly reported.[3] The alternative drugs recommended have been cidofovir and foscarnet, both of which are expensive and have toxic side effects.[4 5]

Leflunomide, (*N*-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide) is an inhibitor of protein kinase activity and pyrimidine synthesis and is an immunosuppressive drug used in rheumatoid arthritis and in rejection in solid organ transplantation. The antiviral activity of leflunomide against CMV was first described by Waldman and colleagues.[6 7] Subsequently there have been a case series and a case report describing the clinical use and benefit of leflunomide for CMV infection.[8 9] In the subsequent section we detail the studies done so far and explore the potential usefulness of this compound for CMV infection and suggest that this is a potentially cost effective new treatment for CMV infection that deserves further study.

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LABORATORY STUDIES

The first report of the potential use of leflunomide for CMV infection came from Waldman and colleagues in 1999 in Columbus, OH.[6 7] Because a number of CMV proteins are phosphorylated, they tested the hypothesis that this agent might exert inhibitory activity against CMV. Plaque assays showed dramatic dose dependent attenuation of production of multiple clinical CMV isolates in leflunomide treated human fibroblasts and endothelial cells, common targets for CMV infection in vivo. Northern blot analysis and immunohistochemical staining showed leflunomide neither interferes with transcription of immediate early or late viral genes, nor with expression of corresponding proteins. CMV specific DNA dot blots and biochemical enzyme assays showed that, in contrast with currently approved anti-CMV drugs, leflunomide exerts no inhibitory effect on the accumulation of viral DNA in infected cells, or on viral DNA polymerase activity. On transmission electron microscopy, this agent seems to act at late stage in virion assembly by preventing tegument acquisition by viral nucleocapsids. They found equivalent inhibitory activity of leflunomide against multidrug resistant CMV isolates. These findings implied that leflunomide, showed potential to attenuate a CMV disease by a novel mechanism of antiviral activity—in contrast with all other anti-CMV drugs currently in use. It does not inhibit viral DNA synthesis, but rather seems to interfere with virion assembly.

In contrast a recent study by Evers and colleagues[10] in Chapel Hill, USA on FK778, an immunosuppressant structurally similar to A771726, the active metabolite of leflunomide, but with a clinically relevant shorter serum half life found that its mode of antiviral action seems to mirror the same as biochemical mechanisms responsible for its immunosuppressive properties: inhibition of protein tyrosine phosphorylation and inhibition of cellular de novo pyrimidine biosynthesis. Initial HCMV mediated activation of the EGF receptor/phosphatidylinositol 3-kinase (PI3-K) pathways and Sp1 and NF-kappaB were partially inhibited by FK778. The second tier (phase) of PI3-K, Sp1, and NF-kappaB induction by HCMV was more sensitive to FK778. Treatment of HCMV infected cells with FK778 prevented the appearance of HCMV proteins some 12–24 hours after infection, and inhibited viral DNA synthesis. The antiviral activity of FK778 was reversed in cell culture by treatment with uridine, consistent with specific inhibition of dihydroorotate dehydrogenase

(DHODH), a required enzyme in the de novo biosynthesis of pyrimidines. As mentioned, these results differ from descriptions of leflunomide acting as an inhibitor of HCMV cytoplasmic capsid formation. They also suggest that that DHODH may be an effective cellular antiviral target.

ANIMAL STUDIES

Zeng and colleagues[11] in Chicago did a mechanistic study of malononitrilamide FK778 and leflunomide in cardiac transplantation and CMV infection in rats. Heart transplants were performed in rats (Brown Norway to Lewis) and treated with varying doses of FK778 or leflunomide for 28 days. At 28 days, at the time of rejection or at the death of the animal, the allograft and other vital organs were obtained for study by light microscopy and immunohistochemistry. In separate experiments, Lewis rats were given sublethal irradiation, inoculated with rat CMV (Maastricht strain), and treated with varying doses of FK778 and leflunomide. In both the transplant and CMV studies, intraperitoneal uridine was given to cohorts or animals receiving FK778 and leflunomide. They found FK778 controls acute rejection and inhibits CMV replication. Toxicity is manifested as anaemia, changes in hepatic and intestinal histology, and mortality. The toxicity but not the immune suppressive or antiviral efficacy, is reduced significantly by exogenous uridine administration. This showed that FK778 has both immune suppressive and antiviral activities, neither of which is entirely dependent on inhibition of pyrimidine synthesis. This suggests that the antiviral activity and a considerable part of the efficacy of the malononitrilamide family of drugs are attributable to activities other than drug induced pyrimidine deficiency.

HUMAN TRIALS

Human trials were first reported from the Christian Medical College, in Vellore, India by John and colleagues[8] who used leflunomide in four consenting renal allograft recipients with symptomatic CMV disease, who were unable to afford gangciclovir and would otherwise remain untreated. It was the first report of efficacy of leflunomide in humans with CMV disease. The patients received loading dose of 100 mg of leflunomide once daily on days 1–3 and then 20 mg once daily for three months. All four patients were followed up three times weekly with physical examination, total leucocyte counts, blood urea, and serum creatinine for a minimum period of six weeks. None of the patients showed drug related adverse events, change in cyclosporine levels, or decreased graft function, except one who developed leucopenia. During follow up all four patients had undetectable viral loads by Q-CMV PCR in an average of about one month and endoscopically confirmed healing of upper gastrointestinal lesions in an average of 1.3 months. These preliminary data suggested that leflunomide therapy for CMV disease is effective and could be used with careful monitoring in allograft recipients who cannot afford intravenous gangciclovir therapy.

Avery and colleagues[9] at the Cleveland Clinic, OH studied an allogeneic bone marrow transplant recipient who developed CMV infection refractory to sequential therapy with gangciclovir, foscarnet, and cidofovir. The patient was ultimately treated with a combination of leflunomide and foscarnet. Both phenotypic and genotypic virological analysis were performed on sequential CMV isolates. The patient's high CMV-DNA viral load became undetectable with leflunomide and foscarnet, but the patient, who had severe graft versus host disease of the liver, died with progressive liver failure and other complications. This suggested that leflunomide has anti-CMV activity, which may be useful in the treatment of multidrug resistant CMV. However, the toxicity profile of leflunomide in patients with underlying graft versus host disease remains to be defined.

DISCUSSION

The search for newer more cost effective treatments for infectious diseases remains a challenge. CMV infection that is especially common in the post-transplant scenario is an important challenge for transplant physicians. Gangciclovir's cost (> $700 per two week course in India) is an important hurdle in developing countries. Leflunomide is cheaper ($33 per course of three months' treatment) and is easily given orally. It works by a novel mechanism inhibiting virion assembly. It also has immunosuppressive properties. It has been shown to be effective in both gangciclovir sensitive as well as resistant cases of CMV infection. Given these considerations we believe that leflunomide is an exiting new drug for CMV infection. Recently the antiviral properties of leflunomide have also been studied in the transplant setting for polyoma virus nephropathy further suggesting a potential role for this agent in certain viral infections.[12] However, hepatotoxicity and teratogenicity are known side effects. The exact dose and duration of treatment for CMV infection, for secondary prophylaxis, and in situations of gangciclovir resistance need further study.

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